

The University of Chicago

CYTOLOGICAL STUDIES OF LILIUM TIGRINUM

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1939

By

JONATHAN J. WESTFALL

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CYTOLOGICAL STUDIES OF LILIUM TIGRINUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 509

JONATHAN J. WESTFALL

(WITH SIXTY-NINE FIGURES)

Introduction

Because it is the only species of *Lilium* reported to possess both diploid and triploid forms, *L. tigrinum* Ker-Gawl. is of special interest. Early investigators reported only the diploid form, but descriptions and figures given in several papers indicate that some of the plants reported as diploids were in reality triploids.

COULTER, CHAMBERLAIN, and SCHAFFNER (13), BELLING (8), and SIANG (36) reported 12 pairs of synaptic mates in the meiotic divisions of the microsporocytes. More recently TAKENAKA and NAGAMATSU (42) reported that this species possessed 36 somatic chromosomes. SATO (32) confirmed this work on the single-flowered variety, and reported that *L. tigrinum* var. *flore-pleno* also has 36 chromosomes. TAKENAKA (41) observed lagging, fragmentation, and other irregularities in the meiotic divisions of the microsporocytes of *L. tigrinum*. In the following year SASS (30) found similar irregularities. MATHER (21) extended the studies to include the additional *tigrinum* varieties *Fortunae giganteum* and *splendens*. Both were found to possess 36 chromosomes. CHANDLER, PORTERFIELD, and STOUT (11) studied chromosome morphology and microsporogenesis in both the diploid and triploid forms. The existence of this self-fertile species of *Lilium*, which proved to be of the diploid type, had previously been reported by PRESTON (25). Chromosome counts of a number of seedlings of the triploid *L. tigrinum* were reported by SATO (33).

Following the account of development of the megagametophyte of *Fritillaria persica*, in which BAMBACIONI (1) described the process wherein four nuclear divisions intervene between the archesporial cell and the egg instead of three, as was formerly supposed, other members of the Liliaceae were examined or reexamined in rapid succession.



BAMBACIONI and GIOMBINI (2) reported a similar process in *Tulipa gesneriana*. This was followed by the work of BAMBACIONI-MAZETTI (3) on *Lilium bulbiferum*, *L. candidum*, and *Tulipa praecox*. Both *T. praecox* and *T. gesneriana* have since been found to be triploids (15). Although her report of the development of the megagametophyte of these two species emphasizes the division and orientation of the nuclei rather than the determination of the exact numbers of chromosomes, some of the illustrative figures included show lagging chromosomes and micronuclei similar to those reported for other triploid plants.

COOPER (12) described the method of development of the megagametophyte in *Lilium henryi* and examined preparations of *L. speciosum*, *L. philadelphicum*, *L. longiflorum* var. *eximium*, *L. regale*, *L. auratum* var. *platyphyllum*, *L. elegans*, *L. martagon*, *L. pardalinum*, and *L. philippinense*. Later SANTOS (29) described the complete process in *L. philippinense*. In all species of *Lilium*, *Fritillaria*, and *Tulipa* examined it was found that in the course of the third nuclear division three of the four nuclei formed as the result of the second meiotic division migrate to the chalazal end of the megagametophyte, and there the three spindles fuse and undergo mitosis. At the same time the lone nucleus at the micropylar end also divides mitotically, so that a second 4-nucleate stage results. Instead of all nuclei being haploid, however, as in the first 4-nucleate stage, the two micropylar nuclei are at the later stage haploid, but the two chalazal nuclei are triploid. One further mitotic nuclear division occurs, resulting in the formation of four nuclei at each end of the megagametophyte. One nucleus from each end, the polar nuclei, move toward the center and unite, the nucleus from the chalazal end contributing $3n$ chromosomes and the nucleus from the micropylar end bringing the total count of the fusion nucleus to $4n$ in the diploid plants. In the process of fertilization one of the male gametes unites with the egg at the micropylar end to form the zygote, while the other unites with the polar nucleus to form the primary endosperm nucleus. This type of megagametophyte development has been designated by SCHNARF (35) as the "*Fritillaria* type," to avoid confusion with the "*Lilium* type" in the older sense.

In other members of the Liliaceae microsporogenesis has been

studied in the triploid forms of *Hyacinthus* by BELLING (6); *Hosta* by YASUI (45); *Hemerocallis* by BELLING (7); *Tulipa* by DARLINGTON (14), NEWTON (24), and WOODS (44); and *Allium* by LEVAN (20).

Among the limited number of reports on the development of the megagametophyte in triploids are those of MORINAGA and FUKUSHIMA (22) on *Oryza* and of SATINA and BLAKESLEE (31) on *Datura stramonium*.

If assortment of chromosomes and distribution of nuclei were the same in the development of the female gametophyte as in the male, studies of both would not be necessary; but the chromosome numbers of the progeny of triploids may be different, depending on whether the triploid is the pollen parent or the seed parent or both. As a general rule extra chromosomes are more often transmitted to the progeny by the female gametes than by the male (14), possibly because of complete failure of growth of the abnormal pollen grains or their failure to compete with pollen grains having the haploid set of chromosomes. Other features, as chromosome lagging, fragmentation, and random assortment—characteristic of autotriploids—as well as the type of division and arrangement of nuclei, make *Lilium tigrinum* particularly interesting for cytological study.

Since SCHAFFNER (34), SASS (30), TAKENAKA (41), and CHANDLER, PORTERFIELD, and STOUT (11) previously reported studies of microsporogenesis in *L. tigrinum*, the present study devotes attention to megasporogenesis and development of the megagametophyte, but makes frequent reference to observations on microsporogenesis.

Material and methods

Anthers and ovaries of *L. tigrinum* var. *splendens* were gathered from plants grown in the experimental garden at the University of Chicago and at Wychwood on Lake Geneva, Wisconsin, during late July and the first two weeks in August of the summer of 1938. The bulbs were originally obtained from Wayside Gardens, Mentor, Ohio.

The iron-aceto-carmin smear method was used in making preliminary studies and in determining the proper stages for fixation of the anthers. In most instances the first meiotic division was found to take place when the buds were about $1\frac{1}{4}$ inches in length, or

about 2-3 days before anthesis. Anther material was fixed at different times during the day over a period of 3 weeks. Ovaries were fixed one day before anthesis and continued at 24-hour intervals until 120 hours after anthesis. The ovaries were cut either longitudinally or transversely to reduce the distance the fixatives had to penetrate. Some of the ovules were dissected out before being fixed.

Smears of the anthers were fixed in Belling's modification of Navashin's and in Carnoy's solutions. Other anthers and the ovaries were allowed to remain in Carnoy's for about 80 seconds, after which they were placed in Navashin's solution. This treatment, as well as using Flemming's or Navashin's alone, gave good fixations. The butyl-alcohol paraffin method of imbedding was used, and sections were cut chiefly at 25-30 μ , although some sections for critical studies of the chromosomes were cut as thin as 6 μ . Either Heidenhain's iron-alum haematoxylin or the crystal violet-iodine method was used in staining all paraffin sections.

Observations were made with either a 10 $\times$ or a 15 $\times$ compensating ocular and a 100 $\times$ fluorite objective. Drawings were made at table level with a camera lucida.

Observations

Critical examination of stages in the development of *L. tigrinum* var. *splendens* indicates that with few exceptions development follows the general plan reported for other species of *Lilium*, *Fritillaria*, and *Tulipa*. In the present paper no attempt is made to reiterate the story of division and orientation of nuclei or to illustrate all stages in developmental sequence. Only those phases in which *L. tigrinum* has been found to differ from other reported species are given particular attention.

In the early prophases of both the megasporocytes and the microsporocytes, chromosomes occur as single or as paired threads. Pairing, first evident at zygotene, is even more apparent at pachytene, when the threads have increased in chromaticity and have contracted somewhat longitudinally. At this stage (fig. 1) two of the synapsed chromosomes are closely associated and coiled throughout long segments, while the third apparently homologous chromosome



FIGS. 1-15.—Prophases and diakinesis in megasporocytes: fig. 1, nucleus at pachytene; fig. 2, late pachytene threads showing type of chromosome association in detail; figs. 3-5, diplotene and early diakinesis showing entangled trivalent groups; figs. 6-15, trivalent associations of single nucleus at diakinesis.

is in contact with the two at irregular intervals (fig. 2). In most instances the two closely synapsed chromosomes appear to have contracted longitudinally more rapidly after pairing than did the third member. Contraction does not seem to be dependent on pairing but is initiated sooner and progresses more rapidly when pairing has taken place. Differential contraction in some instances causes apparent failure of correspondence in chromomere arrangement in homologous chromosomes. At diplotene and early diakinesis (figs. 3-5) the chromatids of each chromosome are plainly visible, and frequently the members of two or more trivalent associations are

TABLE 1

FREQUENCY OF TRIVALENT, BIVALENT, AND UNIVALENT CHROMOSOME ASSOCIATIONS AT DIAKINESIS AND METAPHASE I;
DATA FOR 500 MICROSPOROCTES

ASSOCIATIONS	FRE- QUENCY	PERCENT- AGE FRE- QUENCY
12 trivalents.	293	58.6
11 trivalents, 1 bivalent, and 1 univalent.	155	31.0
10 trivalents, 2 bivalents, and 2 univalents.	39	7.6
9 trivalents, 3 bivalents, and 3 univalents.	11	2.2
8 trivalents, 4 bivalents, and 4 univalents.	2	0.6
Less than 8 trivalents.	0	0.0
Mean no. of trivalents for entire sample.		11.45

entangled so as to produce an apparent secondary association. This is interpreted as a result of chance arrangement of chromosomes preceding pairing, so that true or false interlocking results, rather than as interchange of parts between nonhomologous chromosomes. At diakinesis a single nucleus generally contains three nucleoli of different sizes, although more or less than this number may be present.

Preliminary studies of chromosome associations in the microsporocytes and megasporocytes at diakinesis show no significant differences in the degree of frequency of trivalents, bivalents, and univalents in the two types of cells. Since it is easier to make counts from smear preparations of the microsporocytes, they were used in determining the frequency of different types of associations.

Chromosomes of two or more trivalent groups often remain inter-

meshed throughout diakinesis (figs. 9, 11), but 12 separate trivalent associations occur more frequently.

Trivalents shown in figures 5-15 were drawn from a single nucleus, and in all cases at least one chromosome of each association was in contact with the nuclear membrane. The trivalents are oriented in such a manner that each association, with the exception of those entangled, is well separated from the next, as if repelled. This is not so apparent in single-plane drawings as it is in the material from which they were drawn. In figure 16 the chromosomes are grouped as 11 trivalents, 1 bivalent, and 1 univalent. All chromosomes are grouped as 12 trivalent associations in figure 17. Two univalents are shown apart from the compactly grouped trivalent and bivalent mass in figure 18. Dissociation of some of the trivalents is shown at early metaphase in figure 19.

FIRST MEIOTIC DIVISION

The arrangement of chromosomes at the equatorial plane during metaphase of the first meiotic division seems to be without unusual features (figs. 19, 20). Only occasionally is a univalent left outside the plane toward the poles. The first meiotic division is generally characterized by complete disjunction of all members of trivalent and bivalent associations, although there are two notable exceptions to this general rule. First, chromatids occasionally fail to disjoin at anaphase and tend to adhere near their ends, which are bent out at right angles to the axes of the two chromatids. This type of behavior is shown in the center of figure 21 and in the first of the three bridges of figure 53. The chromatids may eventually disjoin, or non-disjunction may persist in the microsporocytes until after the 2-celled stage. The plainly visible ends of the bridging chromatids and the absence of acentric fragments distinguish this from a more common type of bridge shown to the right of center in figure 21 and to the left of center in figure 22. In figure 23, in addition to four lagging chromosomes between the two organizing telophase nuclei, there is a fragment and an attenuated chromatid bridge which appears to be stretched almost to the breaking point. The frequency of such bridges appears to be about the same in microsporogenesis and megasporogenesis. Of 603 microsporocytes examined, seventy,

or 11.6 per cent, showed the presence of anaphase chromatid bridges. These bridges occasionally persist even after the formation of the



FIGS. 16-23.—Fig. 16, megasporocyte at diakinesis showing 11 trivalents, 11 bivalents, and 1 univalent; fig. 17, nucleus at diakinesis showing trivalent associations and nucleolar attachments; figs. 18-20, metaphase of first meiotic division; figs. 21, 22, anaphase showing chromatid bridges; fig. 23, telophase showing lagging chromosomes and attenuated chromatid bridge.

division figure of the second meiotic division (fig. 25). In figure 30 the two spindles of the second meiotic division are seen unusually close together, still joined by a persisting chromatid connection.

The first meiotic division of the microsporocyte differs from that



FIGS. 24-29.—Fig. 24, metaphase of second meiotic division; fig. 25, early anaphase figures of second meiotic division; figs. 26, 27, anaphase of second meiotic division; fig. 28, metaphase of third nuclear division; fig. 29, 4-nucleate stage following third nuclear division, showing lagging at both micropylar and chalazal ends.

of the megasporocyte in that in the former a cell wall is formed between the two nuclei before the second division. As this cell wall forms it may cut in two any bridges persisting after the first division. More often, however, the bridge prevents the formation of the cell wall at the point through which it passes in connecting the two nuclei. Prevention of cell wall formation is regarded not so much as an inhibitive action on the part of the chromatin material as a mere replacement of the wall-forming cytoplasm at the point where the bridge passes through.

Figure 49 of the division of the microsporocyte is comparable with figure 22 of the megasporocyte. In both figures one of each pair of attached chromatids has completely disjoined, while the other, with the chromatid from which it failed to separate, forms a taut bridge between the separating masses of chromosomes. A sharply bent fragment is shown near the midpoint of each bridge. In addition to the chromosome fragments, lagging chromosomes also occur (figs. 49-51). Figures 49-62 show chromatid bridges and acentric fragments. In the development of the megagametophyte attenuated chromatid bridges are generally broken and may be absorbed in the cytoplasm. Fragments and lagging chromosomes may be absorbed, or they may persist as micronuclei or as granules.

Accurate determination of chromosome distribution at the first and second meiotic divisions was impossible except in a limited number of very favorably oriented cells which showed no lagging. In figure 21 the chromosomes, including those making up the bridge, are evenly divided, 18-18. In figure 22 the distribution is 19-17, including the chromosomes forming the bridge. Counts were made of 27 megasporocytes at the first meiotic division, and the chromosome numbers of the daughter nuclei were found to vary from 12 to 22, excepting 13 and 20. The mean for the distribution was 17.1. These figures are only approximate, because in the division of the megasporocyte no incoming cell wall marks off the cytoplasm into two compartments, and it is therefore impossible to say with which of the daughter nuclei, if with either, the lagging chromosomes should be counted. If they are credited with neither nucleus, or if only figures showing no lagging are counted, the story of chromosome distribution is still incomplete.

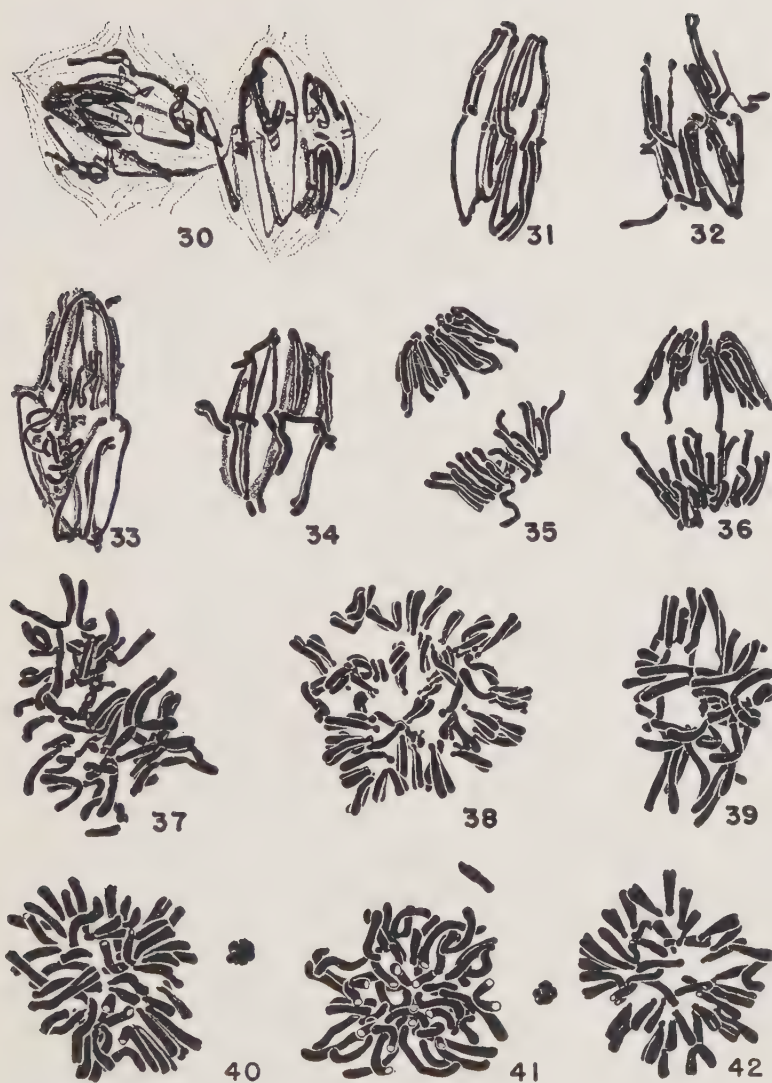
At metaphase of the second meiotic division (figs. 24, 25) few accurate counts could be made because of the manner in which the chromosomes seem to adhere to one another. Further difficulty in making counts was caused by the four chromatids of two adjacent chromosomes with subterminal constrictions being so easily confused with two chromatids of a single chromosome with submedian spindle attachment constriction. With the data from figures that could be counted, however, and from counts at later stages, it seems probable that at the first meiotic division one set of 12 chromosomes goes to one pole and 12 to the other, while the members of the third set are randomly distributed between the two. Of 603 microsporocytes examined, lagging was detected in 47.9 per cent.

SECOND MEIOTIC DIVISION

In the second meiotic division also there is both lagging and bridging. In the lower spindle of figure 26 and in the upper spindle of figure 27 bridging is shown. Approximately midway between the two spindles of figure 27 is shown a lagging chromosome or large fragment in contact with a small accessory spindle. The second meiotic division of the microsporocyte is shown in figure 63. Both bridging and lagging are shown in the lower cell of the two. Typical configurations at metaphase of the second meiotic division are shown in figures 31, 32, and 34. The upper spindle of figure 26 and the lower of figure 27 show the second meiotic division as it more commonly occurs, without bridging and lagging.

In the absence of lagging and bridging the second meiotic division is numerically equational. The effects of lagging, bridging, and fragmentation cause differences in chromosome numbers as great as five in the two nuclei formed as the result of division of a single nucleus. Counts were made of five megasporocytes at the second meiotic division. These limited data are inadequate for drawing conclusions as to the most common assortments, but they serve to illustrate some of the effects of lagging and fragmentation (table 2).

The chromatids, when separated precociously in the first meiotic division, do not divide again in the second. It is possible that owing to unequal distribution of chromosomes the four nuclei formed as a result of the second meiotic division may have different chromosome



FIGS. 30-42.—Fig. 30, division figures of second meiotic division in megasporocyte connected by persisting chromatid bridge; figs. 31-34, late metaphase of second meiotic division; figs. 35, 36, second meiotic division at anaphase; figs. 37-42, chalazal nuclei showing differences in chromosome numbers.

numbers, although this is not usually the case. In the megasporocytes the lagging elements generally roll up in compact masses in the cytoplasm and finally disintegrate or form micronuclei. In the microsporocytes the behavior is much the same except that cell walls may form, splitting off the micronuclei and surrounding cytoplasm to form microcytes. Fragments may persist in the cytoplasm until after the microspores are fully formed.

TABLE 2
DISTRIBUTION OF CHROMOSOMES IN 5 MEGASPOROCYTES AT
SECOND MEIOTIC DIVISION

FIRST SPINDLE			SECOND SPINDLE		
1ST NUCLEUS	LAGGING	2D NUCLEUS	1ST NUCLEUS	LAGGING	2D NUCLEUS
19		19	17	1	16
20		20	16		16
22		22	13		13
17		17	12	4	19
18		18	18		18

THIRD AND FOURTH NUCLEAR DIVISIONS

The somatic number of chromosomes is 36 ($3n$), and if each daughter nucleus should receive equal numbers, the nuclei at the first 4-nucleate stage following the second meiotic division would have 18 chromosomes each. Fusion of three of these nuclei at the chalazal end of the megagametophyte would lead to the formation of a nucleus having 54 chromosomes. The constitution of this chalazal nucleus would be, not $3n$ as in the diploid, but $4\frac{1}{2}n$. These theoretical numbers might be found only in those cases in which there is an equal assortment of chromosomes at both first and second meiotic divisions and no loss through lagging and fragmentation. Counts made at the metaphases of the third nuclear division (fig. 28) showed that the chromosome numbers at both chalazal and micropylar ends vary markedly from the theoretical values. The calculated distribution from random assortment was found by expansion of the binomial. As is shown in tables 3 and 4, both chalazal and micropylar nuclei near or at the upper and lower limits of distribution occur

more frequently than would be expected from random assortment, and correspondingly there are less nuclei with chromosome numbers near the calculated mean than would be expected. If there were no loss of chromosomes in the nuclear divisions of the megagametophyte, the expected number of chromosomes in the chalazal nucleus at the metaphase of the third division might be determined by

TABLE 3
CHROMOSOME NUMBERS IN 150 NUCLEI AT MICROPYLAR END OF MEGAGAMETOPHYTE AT METAPHASE OF FOURTH NUCLEAR DIVISION

CHROMOSOME NO.	FREQUENCY	PERCENTAGE		PERCENTAGE $\frac{\text{OBSERVED}}{\text{CALCULATED}}$
		OBSERVED	CALCULATED ON RANDOM ASSORTMENT	
3.....	1	0.66	0.00	
12.....	6	4.00	0.02	166.60
13.....	13	8.66	0.31	27.93
14.....	19	12.66	1.61	7.86
15.....	26	17.33	5.37	3.22
16.....	28	18.66	12.08	1.54
17.....	25	16.66	19.33	0.86
18.....	11	7.33	22.60	0.32
19.....	7	4.66	19.33	0.24
20.....	2	1.33	12.08	0.10
21.....	2	1.33	5.31	0.24
22.....	5	3.33	1.61	2.06
23.....	0	0.00	0.31	
24.....	3	2.00	0.02	83.33
31.....	1	0.66	0.00	
38.....	1	0.66	0.00	
Mean chromosome no. of observed nuclei.....		16.82		
Mean calculated from random assortment.....		18.00		

counting the number in the micropylar nucleus and applying the formula, $72 - M = C$, where M is the number of chromosomes in the micropylar nucleus and C is the number in the chalazal nucleus. At the metaphase of the fourth division the number of chromosomes would be approximately twice that of the third.

A comparison of the observed numbers of chromosomes in the chalazal nuclei with those calculated from random assortment gave the numbers lacking owing to loss through lagging and fragmenta-

tion in the previous divisions. The number lacking, L , may be obtained for the metaphase of the third nuclear division by the formula, $72 - (M + C) = L$. In a study of 28 megagametophytes in which accurate counts could be made at both micropylar and chalazal ends, it was found that 42.85 per cent of the nuclei showed loss of chromosomes, averaging 8.2 chromosomes, or 11.11 per cent loss per cell. This does not mean that this number of chromosomes was actually left out of the nuclei, but that the number lacking owing to loss is as given. Loss of chromosomes in the first division would appear as a deficiency twice as great at metaphase of the third division, and as a deficiency four times as great at metaphase of the fourth division.

Counts were made of chromosomes in 55 micropylar nuclei at metaphase of the third nuclear division and of 150 at the metaphase of the fourth division. In general the distribution was similar in the two stages, the principal difference being a slight shift toward the lower chromosome numbers in the later stage, as shown by a mean of 16.82 chromosomes at metaphase of the third division as compared with 16.25 for the fourth. The difference is probably due to loss of chromosomes during the third division. In all the megagametophytes examined, with the exception of two, the numbers of chromosomes in the micropylar nuclei ranged from 12 to 24 (table 3, fig. 68). In one of these exceptions one nucleus at the micropylar end had 3 chromosomes and the other had 31. In the other case a restitution nucleus possessing 36 chromosomes apparently was formed, as indicated by the presence of two chalazal nuclei and only one micropylar nucleus.

Chromosome counts were made of 47 chalazal nuclei at metaphase of the third nuclear division and of 41 at metaphase of the fourth division. At metaphase of the third division chromosome numbers ranged from 37 to 60, excepting 39 (table 4, fig. 69). At metaphase of the fourth division the range was from 33 to 60, excepting 34 and 35. The mean number per nucleus at metaphase of the third division was 50.04 as compared with 49.85 for the fourth. Except for this slight difference, the distributions of chromosome numbers were similar in the two stages.

If the third and fourth nuclear divisions in the development of the

megagametophyte were mitotic and equational, as has been reported for other species of *Lilium*, the female gametes of the triploid *L. tigrinum* should receive the number of chromosomes occurring in

TABLE 4
CHROMOSOME NUMBERS IN 47 CHALAZAL NUCLEI OF MEGAGAMETOPHYTE
AT METAPHASE OF THIRD NUCLEAR DIVISION

CHROMOSOME NO.	FREQUENCY	PERCENTAGE		PERCENTAGE $\frac{\text{OBSERVED}}{\text{CALCULATED}}$
		OBSERVED	CALCULATED ON RANDOM ASSORTMENT	
37.....	1	2.12	0.00	
38.....	2	4.25	0.00	
39.....	0	0.00	0.00	
40.....	1	2.12	0.00	
41.....	1	2.12	0.00	
42.....	2	4.25	0.00	
43.....	1	2.12	0.00	
44.....	2	4.25	0.00	
45.....	1	2.12	0.00	
46.....	1	2.12	0.00	
47.....	3	6.38	0.00	
48.....	1	2.12	0.02	88.33
49.....	3	6.38	0.31	20.58
50.....	3	6.38	1.61	3.96
51.....	2	4.25	5.37	0.79
52.....	1	2.12	12.08	0.17
53.....	3	6.38	19.33	0.33
54.....	6	12.72	22.60	0.56
55.....	4	8.51	19.33	0.44
56.....	3	6.38	12.08	0.52
57.....	3	6.38	5.37	1.18
58.....	1	2.12	1.61	1.31
59.....	1	2.12	0.31	6.83
60.....	1	2.12	0.02	88.33

Mean chromosome no. of observed nuclei..... 50.04
Mean calculated from random assortment..... 54.00

the micropylar nuclei preceding the third nuclear division, and likewise the chalazal nuclei at this stage should have the same number of chromosomes as are later contributed to the polar nuclei which subsequently function in the formation of the primary endosperm nucleus. As shown in figure 29, however, lagging in the third and fourth nuclear divisions of both micropylar and chalazal nuclei may

lower the chromosome numbers of nuclei formed by the divisions following meiosis. The nuclei at the micropylar and chalazal ends of figure 29 actually occurred in different cells, but have been placed together for economy of space.

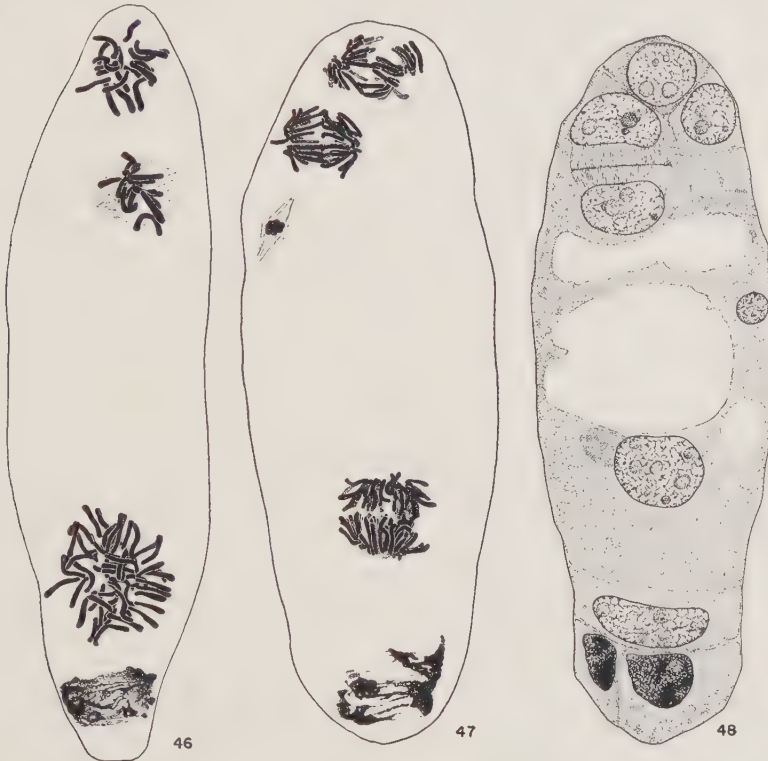
In figures 43 and 44, representing metaphase stages of the fourth nuclear division, compactly knotted lagging chromosomes are



FIGS. 43-45.—Fig. 43, metaphase in fourth nuclear division in development of megagametophyte, showing lagging chromosomes in cytoplasm; fig. 44, metaphase of fourth nuclear division showing widely separated micropylar nuclei; fig. 45, anaphase of fourth nuclear division with micropylar nuclei widely separated.

shown. It is likely that some of these chromatic masses contain more than one chromosome. In figure 45 a chromatic body is shown with a small spindle organized about it. In none of these small spindles have divisions been observed. In figure 48 is a micronucleus which was probably organized from lagging chromosomes lost from the other nuclei. At metaphase of the fourth division the usual

orientation of the four nuclei is as shown in figure 46, with two small nuclei at the micropylar end and two larger ones at the chalazal end. This orientation is generally maintained effectively by the formation of one or more large vacuoles between the two sets of



FIGS. 46-48.—Fig. 46, metaphase of fourth nuclear division as it usually appears; fig. 47, anaphase of fourth nuclear division showing apparently amitotic division of lower chalazal nucleus and a chromatic body in the cytoplasm; fig. 48, 8-nucleate megagametophyte showing egg and two synergids at micropylar end, two polar nuclei and micronucleus toward center, and three antipodal cells at chalazal end.

two nuclei each. Frequently, however, vacuoles may form between the two micropylar nuclei, in which case one is crowded away from the other and forced down into the region of the chalazal nucleus (figs. 44, 45). Still less frequently all four nuclei may be forced by vacuole formation into either the micropylar or the chalazal end.

The lower chalazal nucleus divides apparently amitotically in the fourth division (figs. 44-47), while the upper chalazal nucleus forms a well-spaced metaphase plate (fig. 46). It was at this stage that counts were made. The 8-nucleate megagametophyte is represented in figure 46, in which a small micronucleus is also shown.

Discussion

It has been reported that the diploid *Lilium tigrinum* sets seed readily when self-pollinated, but that the triploid form rarely does (32). STOUT (38, 39), who collected over 200 types of *L. tigrinum* which produced no seeds following self-, close-, and inter-pollination, states that the single-flowered clones of this species known previous to 1932 have been completely fruitless to cross- and self-pollination. SATO (33) reported chromosome counts of the progeny of self- and inter-pollinated plants of *L. tigrinum* and found a wide variation in chromosome numbers, ranging from 24 to 39, excepting 31. He concluded that these seedlings must have resulted from the fusion of gametes with irregular numbers of chromosomes produced by an autotriploid mother plant. From a consideration of the chromosome numbers of the progeny, he presumed the existence of gametes ranging from 12 to 27, with 12 and 14 chromosomes in nuclei occurring most frequently. He also reported the occurrence of fragments, and postulated a structural change in some of the chromosomes.

The plants used in the present investigation have been observed for a number of years and have never been seen to produce capsules following self- or inter-pollination. That sterility is due principally to self-incompatibility in the clones of the triploid *L. tigrinum* rather than to failure of formation of functional gametes is shown by the production of both capsules and viable seeds when cross-pollinated with other species, using the triploid *L. tigrinum* as seed parent in some instances and as pollen parent in others. Table 5 lists successful crosses taken from several different reports.

No cytological work has been reported on any of the *L. tigrinum* hybrids, but in view of the present investigation it seems probable that such hybrids are likely to be subject to irregularities in meiotic divisions and to show considerable variations in somatic numbers. Data given by CHANDLER, PORTERFIELD, and STOUT (11) on the

division of the microsporocyte, and additional data obtained in the present investigation on the divisions of both the microsporocytes and the megasporocytes of *L. tigrinum*, indicate that a set of 12 chromosomes passes to each pole in the first meiotic division, and that members of the third set are more or less randomly distributed

TABLE 5
LILIUM TIGRINUM CROSSES

LILIUM SEED PARENT AND POLLEN PARENT	INVESTIGATOR AND DATE
tigrinum×maximowiczii.....	Preston (25), 1925
tigrinum var. splendens×maximowiczii.....	Stout (38), 1926
tigrinum×sutchusense.....	Beal (4), 1937
tigrinum×warleyense.....	Stout, 1926
tigrinum×willimottiae.....	Preston (26), 1933
tigrinum var. splendens×willimottiae.....	Preston, 1933
tigrinum×davidii.....	Beal, 1936
tigrinum×amabile.....	Preston, 1933
tigrinum×elegans.....	Stooke (37), 1937
tigrinum×elegans wallacei.....	Stooke, 1937
tigrinum×pseudotigrinum.....	Stout, 1926
tigrinum×tigrimax.....	Preston, 1933
tigrinum var. splendens×tigrimax.....	Preston, 1933
tigrinum var. splendens×auratum.....	Beal, 1937
tigrinum var. splendens×batemanniae.....	Beal, 1936
tigrinum var. splendens×regale.....	Beal, 1936
tigrinum var. splendens×superbum.....	Beal, 1936
tigrinum var. splendens×leichtlinii.....	Beal, 1937
speciosum×tigrinum.....	Preston, 1933
bulbiferum croceum×tigrinum var. splendens..	Stooke, 1937
henryii×tigrinum.....	Preston, 1933
henryii×tigrinum var. splendens.....	Beal, 1936
speciosum×tigrinum.....	Preston, 1933
davidii×tigrinum (2n=24) var. fortuneae.....	Preston, 1933
maximowiczii×tigrinum.....	Preston, 1933
regale×tigrinum.....	Preston, 1933
regale×tigrinum var. splendens.....	Beal, 1937
superbum×tigrinum var. splendens.....	Beal, 1936

between the two poles. Elimination of chromosomes causes a general shift toward the lower numbers from the distribution expected on random assortment. The result is that nuclei with n number of chromosomes or with $n + 1$ or $n + 2$ occur with much greater frequency than would be expected from random assortment. That loss of chromosomes through lagging is not entirely responsible for the greater than expected frequencies of nuclei with low chromosome

numbers is shown by the occurrence of many more nuclei than is expected at the upper limit of distribution. Even after having the chromosome numbers lowered by lagging and fragmentation, nuclei at or near the diploid end of the distribution occur in greater than expected frequencies (figs. 68, 69; tables 3, 4).

After allowance is made for the general shift to the left, the distribution approaches that expected from random assortment, except that fewer nuclei are found at or near the mean and more at or near the haploid and diploid limits than expected. A departure from calculated distribution similar to this has been reported for several triploids. BELLING (5) for *Canna*, DERMEN (16) for *Petunia*, BELLING and BLAKESLEE (9) and SATINA and BLAKESLEE (31) for *Datura* have reported that triploid plants of these genera show chromosome distribution at meiosis similar to that here found for *L. tigrinum*.

In later stages of development in the male gametes in triploids a wide variation in chromosome numbers has been reported. SATINA and BLAKESLEE on *Datura*, DARLINGTON (15) on *Hyacinthus*, LEVAN (20) on *Allium*, CAPINPIN (10) on *Oenothera*, and NAGAO (23) on *Narcissus* reported nuclei with all chromosomes, ranging from n to $2n$. In most of the investigations nuclei with lower chromosome numbers were found to be more numerous than those at the other limit of distribution. Not in all instances were the numbers of nuclei at or near the limits of distribution greater than expected.

The distribution of chromosomes at the metaphases of both the third and fourth nuclear divisions is similar in micropylar and chalazal nuclei, except that the effects of lagging and fragmentation are trebled in the chalazal nuclei, and as a result the shift to the lower chromosome numbers is more marked than in the micropylar nuclei (figs. 68, 69). Lagging in the nuclear divisions following meiosis has not been reported in previous investigations. In *L. tigrinum* such lagging occurs rather infrequently as compared with that in the meiotic divisions, and is possibly related to the chromosome unbalance and the existence of dicentric chromosomes and fragments. Actual counts were not made of chromosomes in the female gametes, but numbers at the metaphase of the fourth nuclear division should represent those counts rather accurately, since numbers would be modified so slightly in the fourth division.

Differential assortment of chromosomes of the type favoring the production of haploid and diploid gametes, as it occurs in *L. tigrinum*, may be explained if it is found that certain homologous chromosomes tend to pass together to the same pole. Behavior of this type has been reported in *Drosophila* by STURTEVANT (40) and in *Rosa* by HURST (19), but neither of these cases is comparable with that of *L. tigrinum*. In *Rosa*, chromosomes which failed to pair went together to the same pole. As shown in table 1, there is almost complete trivalency in the triploid *L. tigrinum*, indicating that the three sets of chromosomes making up the somatic number are very similar. There is, nevertheless, evidence that one set of 12 chromosomes may differ from the other two in regard to the structure of some of its members. Failure of correspondence in all chromomeres of a set of three homologous chromosomes has been mentioned. In previous investigations SATO (33) and CHANDLER, PORTERFIELD, and STOUT (11) also reported such apparent lack of strict homology in the prophases of the microsporocyte. They gave no definite suggestion as to the cause of the irregularity.

In the present investigation at early diakinesis of the megasporocyte three chromosomes were observed associated as shown in figure 66*u*, and as interpreted in figure 66*v*. Such configuration may result from a cross-over in an inverted segment distal to the centromere in the first of the three chromosomes. The break in the chromosome is supposed to have occurred at the point *x*, and an apparent lack of symmetry at this point—which corresponds in position to the end of the second chromosome—gives additional evidence in support of this supposition. The occurrence of chromatid bridges and fragments at the anaphase and telophase of both the first and second meiotic divisions adds confirmatory evidence for the existence of an inverted segment in at least one of the chromosomes.

The relationship of bridging and fragmentation to crossing-over in an inverted segment distal to the centromere of one of the chromosomes is shown in figure 66, columns I, II, III, and IV. In the first three columns only two of the three homologous chromosomes are considered. In column I is diagrammed the possible sequence of events after the formation of one chiasma at pachytene (*c*), followed by bridge formation and fragmentation in metaphase and anaphase (*d*, *c*). In column II a similar sequence is shown following formation

of two chiasmata. The final configuration is the same in both cases. The acentric fragment may vary in length, depending on the distance from the end of the chromosome at which the chiasma in the inverted portion forms. Nuclei showing bridges and acentric fragments which may have been formed according to the plan of columns I and II are shown in figures 21, 22, 23, 30, and 49-60.

In column III of figure 66 is shown a complementary cross-over, resulting in the formation of two bridges and two acentric fragments. Cases of double bridge formation are shown in figures 53 and 54, and in *D* and *E* of figure 67. In column IV of figure 66 and in figure 67 are presented types of separation possible when three homologous chromosomes are associated as shown in figure 66*u*. It is probable that the two chromosomes joined by a chromatid connection tend to move together to the same pole in the first meiotic division, in which case the bridge is not evident until the second meiotic division. It may then occur in one of the two dividing nuclei (figs. 26, 27, 63).

If the two centromeres of the bridge-forming chromosome should always pass to opposite poles in the second meiotic division (fig. 67*J, K*), the ratio of bridges in the first and second meiotic divisions would form an index measuring the degree of differential or random assortment at the first division. But if the two centromeres should pass to the same pole, as in figure 67*L*, which is probably more likely, then the number of bridges appearing stretched from pole to pole in the second meiotic division would represent only a portion of the dicentric chromosomes actually present. In order to compute the degree of differential or random assortment in the first meiotic division, the percentage of bridges apparent in the second meiotic division should be multiplied by the ratio of dicentric chromosomes present to those apparent as bridges. For example, following random assortment of the two centromeres of the dicentric chromosome at the second meiotic division, the ratio of dicentric chromosomes present to those apparent as bridges would be 2 to 1. If, as is likely, the assortment is differential in favor of the two centromeres passing to the same pole, the ratio would be more than 2 to 1. Counts of chromosomes in the microsporocytes show an average of 17 per cent bridging in the second division as compared with 11.6 for the

first. If the percentage of bridges apparent in the second division is multiplied by two to allow for random assortment, the ratio of



FIGS. 49-65.—Figs. 49, 50, first meiotic division of microsporocytes, showing lagging and bridging; figs. 51, 52, 55, bridging and acentric fragments in microsporocytes; figs. 53, 54, double bridging; figs. 56, 62, bridges persisting at metaphase of second meiotic division; figs. 57-61, late anaphase and telophase of first meiotic division, showing bridging and acentric fragments; fig. 63, lagging chromosomes and bridging in second meiotic division; figs. 64, 65, microspores showing chromatic masses in cytoplasm.

bridges in the first meiotic division is 11.6 to 2×17 , or 11.6 to 34. This means that two chromosomes attached by a dicentric chroma-

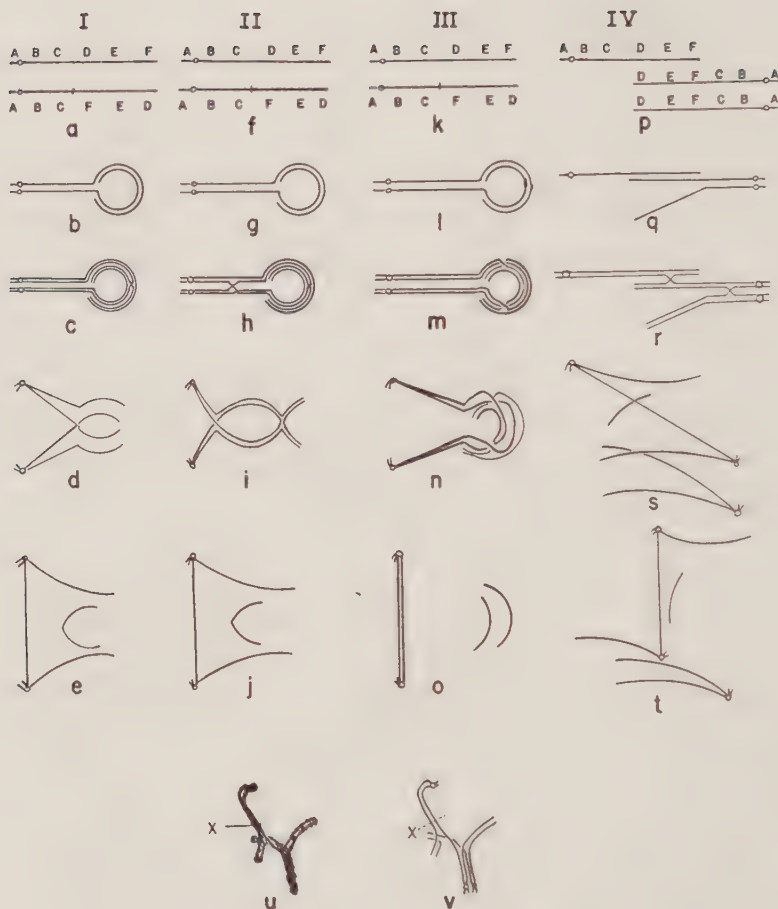


FIG. 66.—Diagrammatic representation of bridging as result of cross-over in inverted segment. Column I: *a*, two chromosomes showing reversal in segment of lower of the two; *b*, zygotene; *c*, pachytene with one chiasma in inverted portion; *d*, *e*, chromatid bridge and acentric fragment. Column II, sequence following formation of two chiasmata. Column III, double bridging following complementary cross-over. Column IV, sequence of events expected accompanying separation of trivalent chromosomes, one of which possesses inverted segment; *u*, observed trivalent association diagrammed in *v*, on which column IV is based.

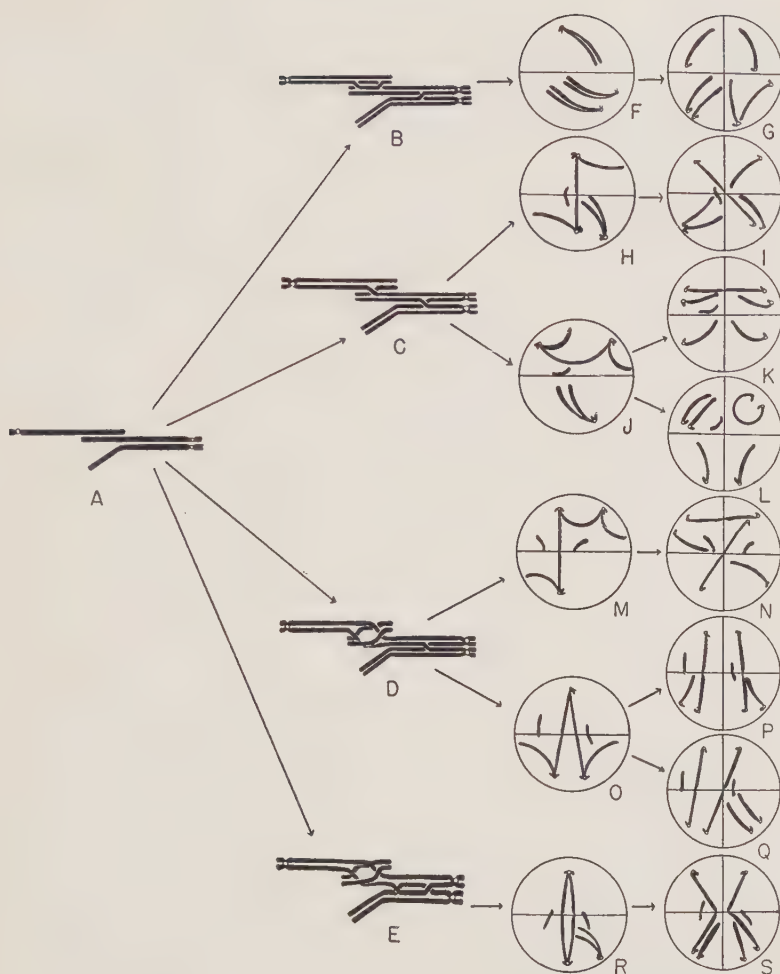


FIG. 67.—Possible meiotic distribution of three homologous chromosomes, one of which shows inverted segment: *A*, three homologous chromosomes arranged as in fig. 66, column IV; *B, F, G*, reciprocal crossing-over in which no bridges are formed; *C, H, I*, bridging at first meiotic division persisting until after the second; *C, J, K*, bridging at second meiotic division; *C, J, L*, no bridge formation following passage of the two centromeres of dicentric chromosome to same pole at both first and second meiotic divisions; *D, M, N, O, P, Q*, some possible arrangements of chromosomes following double bridge formation joining three chromosomes; *E, R, S*, double bridging resulting from complementary cross-over involving only two homologous chromosomes.

tid at the first meiotic division pass together to the same pole approximately three times more often than to opposite poles. If there is a similar differential assortment of centromeres of the dicentric chromosomes at the second meiotic division, only those whose centromeres pass to opposite poles are apparent as bridges, and the

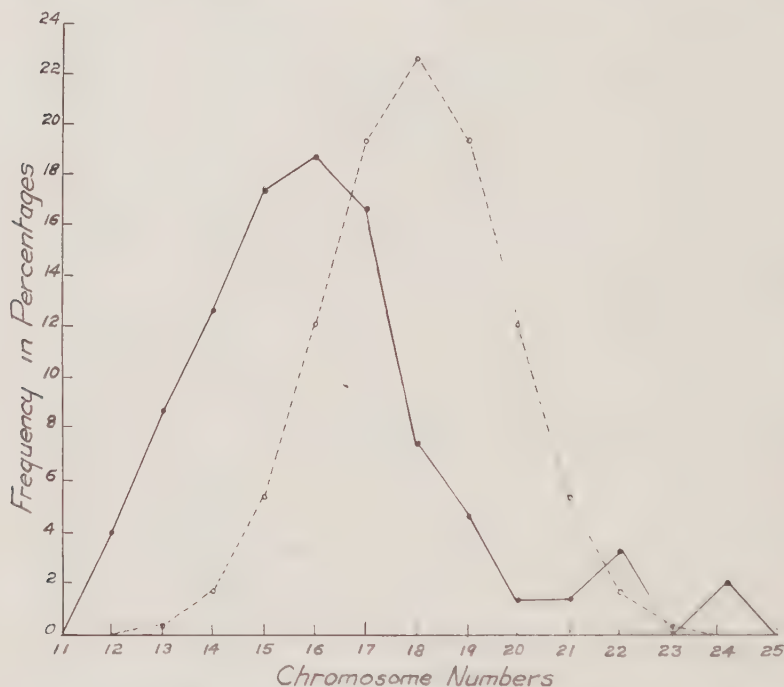


FIG. 68.—Graph showing distribution of chromosome numbers in 150 nuclei at micropylar end of megagametophyte, at metaphase of fourth nuclear division. Broken lines indicate distribution calculated from random assortment; solid lines represent observed frequencies.

index of differential assortment at the first meiotic division is considerably more than 3 to 1.

It is also possible that infrequently three homologous chromosomes become connected by two chromatid bridges (fig. 67*D*, *M*, *O*). Possible results of random assortment of the centromeres of the bridge-forming chromatids are shown in figure 67*N*, *P*, *Q*. More often the centromeres may all pass to the same pole, since all are

connected by bridges, and this would result in a decided differential assortment favoring formation of haploid and diploid nuclei and decreasing the number near the mean expected from random assortment. The nuclei with smaller chromosome numbers formed in this way would be non-functional as gametes, since they would not have a set of 12 different chromosomes. Half of the nuclei formed following the formation of two bridges (fig. 67*R, Q, S*) would also have an incomplete set of entire chromosomes. Although the oc-

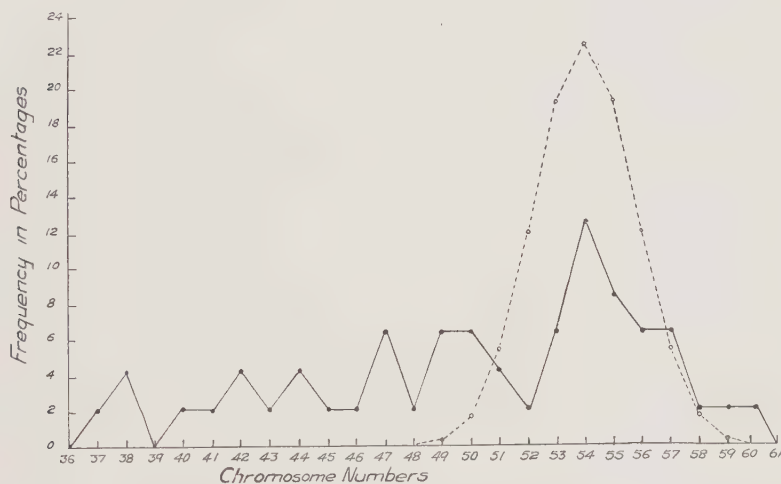


FIG. 69.—Graph showing distribution of chromosome numbers in 47 chalazal nuclei of megagametophyte, at metaphase of third nuclear division. Broken lines indicate distribution calculated from random assortment, solid lines represent observed frequencies.

currence of three homologous chromosomes joined by chromatid bridges may result in a more marked differential assortment in the particular cell in which it occurs than does the occurrence of two chromosomes so joined, the latter condition is so much more frequent that it probably influences differential assortment as a whole more. The numbers of haploid and diploid nuclei resulting from differential assortment would be approximately equal were it not for lagging and fragmentation, which decrease the number of nuclei with high chromosome counts and increase the number with low counts. It is therefore probable that the number of diploid progeny

of a cross between *L. tigrinum* and a diploid form would exceed that expected. Selective growth favoring haploid gametes at fertilization would also tend to produce diploid progeny. Evidence that the proportion of diploid plants resulting from triploid $\times$ diploid crosses exceeds that expected from random assortment has been presented by BELLING and BLAKESLEE (9) in their work with *Datura* and by VAN OVEREEM (43) with *Oenothera*.

The presence of an inverted segment in at least one of its 36 chromosomes places *L. tigrinum* var. *splendens* in the structural hybrid class, notwithstanding the high degree of trivalency at diakinesis. Evidence for structural changes in chromosomes has previously been reported for several species of *Lilium*. RICHARDSON (27) reported inversions and duplications in the hybrid *L. martagon* $\times$ *L. hansonii*. Failure of pairing has been reported for other species by HALL and MATHER (18), BELLING (8), SANSOME and LACOUR (28), and SATO (33). It is possible that many of the recognized species of *Lilium*, including *L. tigrinum*, are of hybrid origin. The prevalence of structural hybridity, although not conclusive evidence for interspecific hybridity, is at least of suggestive significance.

Bridging and fragmentation have been reported in structural hybrids of other genera. Recently WOODS (44), in an investigation of the triploid tulip Inglescomb Yellow, reported both bridging and fragmentation. As is true of *L. tigrinum*, the Inglescomb Yellow tulip showed a high degree of trivalency at diakinesis. In another recent investigation EMSWELLER and JONES (17) reported bridging in a hybrid between *Allium cepa* and *A. fistulosa*.

A close parallelism is evident between meiotic irregularities associated with structural hybridity due to inversion and irregularities commonly reported in triploids at meiosis. Among these irregularities are formation of univalents, which results in lagging chromosomes and subsequent production of micronuclei and microcytes. A second case of parallelism occurs in the type of departure from random assortment of chromosomes. The formation of bridges has also been reported in both types. These similarities in behavior suggest that structural hybridity may occur in triploids more often than has been supposed.

Summary

1. Development of the megagametophyte in *Lilium tigrinum* var. *splendens* follows the general plan reported for the other members of the genus and for *Fritillaria* and *Tulipa*.

2. At metaphase of the first meiotic division 36 chromosomes generally occur in 12 trivalent groups, although bivalents and univalents may occur.

3. Distribution of chromosomes at the first meiotic division approaches random assortment, except that there are marked increases toward the $1n$ and $2n$ limits of distribution.

4. The chromatids of lagging chromosomes may separate precociously following the first meiotic division, after which they may pass tardily to the poles or remain in the cytoplasm between the two nuclei.

5. Lagging chromosomes and fragments eliminated from nuclei may disintegrate or persist as micronuclei.

6. Elimination of chromosomes through lagging and fragmentation in the meiotic divisions and to a less marked degree in the divisions following meiosis produces a general shift in the distribution toward the lower chromosome numbers.

7. Lagging chromosomes and bridging occur in the divisions following meiosis in the development of the megagametophyte.

8. The occurrence of chromatid bridges and fragmentation of chromosomes at meiosis results from crossing-over in an inverted segment of one of the chromosomes.

9. Crossing-over in an inversion results in the formation of dicentric chromosomes at meiosis in a triploid and thus favors the formation of haploid and diploid gametes.

10. There is close parallelism between meiotic irregularities found in structural hybrids and irregularities common to triploids.

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LITERATURE CITED

1. BAMBACIONI, V., Come avviene in *Fritillaria persica* L. lo sviluppo del gametofito femminile e l'aumento dei cromosomi nella regione chalazale. Rend. Acc. Lincei Roma, cl. fis. Mat. Nat. Ser. 6:544-546. 1928.
2. BAMBACIONI, V., and GIOMBINI, A., Sullo sviluppo del gametofito femminile in *Tulipa gesneriana* L. Ann. di Bot. 18:373-386. 1930.
3. BAMBACIONI-MEZETTI, V., Neue ricerche sull'embriologia delle Gigliaceae. Ann. di Bot. 19:365-382. 1932.
4. BEAL, J. M., Unpublished work.
5. BELLING, J., The behavior of homologous chromosomes in a triploid *Canna*. Proc. Nat. Acad. Sci. 7:197-201. 1921.
6. ———, The distribution of chromosomes in pollen grains of a triploid hyacinth. Amer. Nat. 58:440-446. 1924.
7. ———, Chromosomes of *Canna* and *Hemerocallis*. Jour. Hered. 16:465-466. 1925.
8. ———, Nodes and chiasmata in the bivalents of *Lilium* with regard to segmental interchange. Biol. Bull. 54:465-470. 1928.
9. BELLING, J., and BLAKESLEE, A. F., The assortment of chromosomes in triploid *Daturas*. Amer. Nat. 56:339-346. 1922.
10. CAPINPIN, J. M., Studies on the genetics and cytology of triploid *Oenotheras*. Cytologia 4:355-426. 1933.
11. CHANDLER, C., PORTERFIELD, W. M., and STOUT, A. B., Microsporogenesis in diploid and triploid types of *Lilium tigrinum* with special reference to abortions. Cytologia, Fujii Jub. Vol. pp. 756-784. 1937.
12. COOPER, D. C., Macrosporogenesis and development of the embryo sac in *Lilium henryi*. Bot. Gaz. 97:346-355. 1935.
13. COULTER, J. M., CHAMBERLAIN, C. J., and SCHAFFNER, J. H., Contribution to the life history of *Lilium philadelphicum*. Bot. Gaz. 23:412-452. 1897.
14. DARLINGTON, C. D., The origin and behavior of chiasmata. III. Triploid *Tulipa*. Cytologia 4:1-15. 1932.
15. ———, Recent advances in cytology, 2d ed. Churchill Ltd., London. 1937.
16. DERMEN, H., Polyploidy in *Petunia*. Amer. Jour. Bot. 18:250-261. 1931.
17. EMSWELLER, S. L., and JONES, H. A., Crossing-over fragmentation, and formation of new chromosomes in an *Allium* species hybrid. Bot. Gaz. 99:729-772. 1938.
18. HALL, D. A., and MATHER, K., The chromosomes of *Lilium*. Roy. Hort. Soc. Lily Yearbook. 1934.
19. HURST, C. C., The mechanics of creative evolution. MacMillan, New York. 1932.
20. LEVAN, A., Cytological studies in *Allium*. III. *Allium carinatum* and *Allium oleraceum*. Hereditas 18:101-114. 1933.
21. MATHER, K., Meiosis in *Lilium*. Cytologia 6:354-380. 1935.
22. MORINAGA, T., and FUKUSHIMA, E., Cyto-genetical studies in *Oryza sativa*

- L. II. Spontaneous autotriploid mutants in *Oryza sativa* L. Jap. Jour. Bot. 7:207-225. 1935.
23. NAGAO, S., Distribution of chromosomes in pollen grains in certain triploid and hypertriploid *Narcissus* plants. Jap. Jour. Genet. 11:1-5. 1935.
24. NEWTON, W. C. F., Chromosome studies in *Tulipa* and some related genera. Jour. Linn. Soc. Bot. 47:339-354. 1927.
25. PRESTON, I., Lilies of 1924 in Ottawa, Canada. Gard. Chron. 77:270-271. 1925.
26. ———, Hybridization of lilies. Roy. Hort. Soc. Lily Yearbook. 1933.
27. RICHARDSON, M. M., Structural hybridity in *Lilium martagon album* × *L. hansonii*. Jour. Genetics 32:411-450. 1936.
28. SANSOME, MRS., RICHARDSON, E., and LACOUR, L., The chromosomes of *Lilium*. III. Roy. Hort. Soc. Lily Yearbook 3:40-45. 1934.
29. SANTOS, J. K., Macrosporogenesis in *Lilium philippinense* Baker. Cytologia, Fujii Jub. Vol. pp. 822-835. 1937.
30. SASS, J. E., Chromosome fragmentation in *Lilium tigrinum*. Amer. Nat. 68:471-475. 1934.
31. SATINA, S., and BLAKESLEE, A. F., Chromosome behavior in triploid *Datura*. II. The female gametophyte. Amer. Jour. Bot. 24:621-627. 1937.
32. SATO, M., Chromosome studies in *Lilium*. Bot. Mag. Tokyo 46:68-88. 1932.
33. ———, Chromosome variations in the progeny of triploid *Lilium tigrinum*. Cytologia, Fujii Jub. Vol. pp. 1056-1061. 1937.
34. SCHAFFNER, J. H., Chromosome reduction in microsporocytes of *Lilium tigrinum*. BOT. GAZ. 41:183-191. 1906.
35. SCHNARF, K., Contemporary understanding of embryo-sac development among angiosperms. Bot. Rev. 2:565-585. 1936.
36. SIANG, H., Structure of somatic chromosomes of *Lilium tigrinum*. La Cellule 41:163-173. 1932.
37. STOOKE, J. E. H., Roy. Hort. Soc. Lily Yearbook 6:137. 1937.
38. STOUT, A. B., The capsules, seeds and seedlings of the tiger lily, *Lilium tigrinum*. Bull. Torr. Bot. Club 53:269-278. 1926.
39. ———, Sterilities in lilies. Roy. Hort. Soc. Lily Yearbook. 1933.
40. STURTEVANT, A. H., Preferential segregation in triplo-IV females of *Drosophila melanogaster*. Genetics 21:444-466. 1936.
41. TAKENAKA, Y., On the irregular meiotic division in the pollen mother cell of *Lilium tigrinum* Ker-Gawl. Bot. Mag. Tokyo 47:125-131. 1933.
42. TAKENAKA, Y., and NAGAMATSU, T., On the chromosomes of *Lilium tigrinum* Ker-Gawl. Bot. Mag. Tokyo 44:386-391. 1930.
43. VAN OVEREEM, C., Über Formen mit abweichender Chromosomenzahl bei *Oenothera*. Beih. Bot. Centralbl. 38:73-113. 1921.
44. WOODS, M. W., Meiotic studies in triploid *Tulipa* with special reference to bridging and fragmentation. BOT. GAZ. 99:103-115. 1937.
45. YASUI, K., Cytological studies in diploid and triploid *Hosta*. Cytologia 6:484-491. 1935.

